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IMMUNITY OF GUINEA-PIGS TO DIPHTHERIA TOXIN AND ITS EFFECT UPON THE OFFSPRING.

Part 1.

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Since the time Ehrlich(1) established on a scientific basis many of the main facts regarding the transmission of immunity as exhibited in mice to the vegetable poisons, abrin, ricin and robin, many investigators have concerned themselves with the subject of transmission of immunity to various poisons and bacterial infections. Ehrlich first showed conclusively that the immunity transmitted from the mother was of a passive nature, due to the transference of the antibodies from the body fluids of the mother to the foetus through the placenta, and also in the case of the mouse by means of the milk during lactation. He found that the immunity thus transmitted disappeared some three months after birth ; no trace of immunity was detected in the next generation ; neither was an immunised father capable of transmitting immunity to his offspring. Ehrlich concluded from these results that there exists no true hereditary transmission of immunity in mice, a conclusion further supported in both mice and guinea-pigs immunised against tetanus(2).

These results have in the main been confirmed by all later observers. The fact that the young of mice become immunised through the milk is exceptional and not generally shown by other animals experimented upon. Carrière(3) obtained results in guinea-pigs inoculated with products of the tubercle bacillus which show that not only is immunity in the strict sense of the term not transmitted to the offspring but on the contrary a condition of increased susceptibility and a general lowered condition of vitality of the offspring resulted.

Lustig(4) similarly demonstrated that fowls inoculated with abrin produce offspring of less than normal resistance to this poison.

Anderson(5) appears to be the first to have shown that the young of guinea-pigs treated with a mixture of diphtheria toxin and antitoxin exhibit immunity to diphtheria toxin, no such immunity being shown in the young of those treated with toxin only. The immunity was not transmitted by all mothers treated with the toxin-antitoxin mixture, and this he compared with the fact that horses respond very differently to diphtheria toxin in their capacity to produce antitoxin. Grandchildren of immune mothers were not more resistant than normal. Theobald Smith(6) arrived independently at results confirming those of Anderson.

In a second paper Theobald Smith(7) arrived at the following conclusions:

Active immunity to a relatively high degree can be produced in guinea-pigs by the injection of diphtheria toxin-antitoxin.

Neutral mixtures are less effective in producing immunity than mixtures which contain excess of toxin capable of giving rise to local lesions.

We have been engaged on observations on somewhat similar lines for a number of years. We have worked on a larger aggregate of material, and by methods differing in some important particulars from those adopted by the above observers. Although, therefore, our results to a large extent correspond with those which have been published meanwhile by others independently engaged with the question, we think it desirable to place them on record. We have, however, delayed publication until various other questions which had arisen in connection with certain phenomena occurring in the second and third generation could be followed out.

Our method of determining the degree of immunity conferred on the offspring differed from that of Theobald Smith who ascertained the L+ dose on animals of a definite age, while our results were based upon the effect of the injection of toxin only as soon as the young had reached the weight of 250 grams. The degree of immunity depending presumably upon the quantity of circulating antitoxin is more accurately expressed by the neutralising value in terms of the volume of toxin (indicating the number of binding units) rather than the number of fatal doses tolerated. In all cases tabulated the same toxin (98 A) was use throughout.

The seasonal variation(8) of susceptibility of guinea-pigs which affects so markedly the apparent minimal fatal dose of a toxin is barely notice-

able in testing neutralising values. In the same way the volume of toxin tolerated by immune young (*i.e.* neutralised by their antitoxin) shows no great variation at different times of the year except in those cases where the volume of toxin tested was very close to the normal fatal dose. In testing the immunity of a pig only one test is permissible, so that it is seldom possible to determine accurately the immunity value, beyond saying that it is above or below a certain value. When there are three or four pigs in one litter, the first pigs reaching the standard weight can be used as an index for the value to test for in the other pigs of the same litter, and so a more accurate immunity value can be obtained.

In tabulating the results of immunity conferred upon their young by immunised does the degree of immunity is measured by the largest dose of toxin tolerated by any of the offspring, or if the doses given were in all cases too high and none of the young survived, the immunity is taken to be less than the lowest dose that killed. In practice it was found generally unworkable to investigate a particular point by performing definite experiments avoiding all other variables. The possibilities of the injected pigs dying before any young were born, or of the young dying before they reached standard weight, or of there being an insufficient number of young to fix the degree of immunity with any accuracy, were so great that it was found best to obtain our main results by keeping isolated and breeding from a large number of guinea-pigs that survived injections of toxin or toxin and antitoxin mixture given in the course of the regular laboratory routine rather than by specially injecting a limited number of pigs and endeavouring to breed from them. The results obtained in testing the immunity of the offspring from these pigs could then be tabulated. In each set of tables where the results are divided according to a particular variable the other variables are ignored. When it would appear that the results of one variable would overwhelm those of another, individual cases are dealt with.

The first part of our results deals only with the first generation of the young from injected does and normal bucks.

A. *Immunity transmitted to young by mother injected with various mixtures of toxin and antitoxin.*

The variables upon which the immunity of the young may depend are:

1. Nature of the injection received by the mother.
2. Individual differences of different mothers.

3. Time elapsing between injection of mother and birth of young.
4. Age of young when tested for immunity.
5. Time of year and climatic conditions when test was made.
6. Individual differences in young.

These variables are dealt with individually below:

1. *Nature of injection received by mother.*

1 *a*. The constitution of the mixture and the degree to which the mother was affected may be indicated by the local reaction produced by the injection. In Table I the results are divided according to whether the local reaction produced was nil, small or large. The results of immunity conferred by the mother are indicated by the largest dose of toxin tolerated by any of their young, or if none of the young survived the dose given, then the lowest dose that killed is recorded, provided that death occurred within 10 days of the test and that the test was made upon young born within 12 months of the injection of the mother. It should be remembered that in these tables each mother is represented only once although tests may have been made upon many of its offspring.

TABLE I.

Results of immunity of young divided according to the local reaction produced by the immunising mixture when injected into the mother.

		Size of local reaction in mother		
		Nil	Small	Large
Lowest dose killing young	Under 0·008 c.c. ...	4	2	0
	0·008 and under 0·010 c.c.	9	2	0
	0·010 ,, 0·020 c.c.	8	1	1
	0·020 ,, 0·030 c.c.	2	0	0
Highest dose tolerated by young	0·008 and under 0·010 c.c.	3	0	0
	0·010 ,, 0·020 c.c.	6	3	6
	0·020 ,, 0·030 c.c.	4	1	3
	Over 0·030 c.c. ...	3	2	2

To summarise these results it is necessary to consider the proportion of does whose young tolerated a particular dose or more of toxin to those whose young were killed by that or a lower dose of toxin. Thus in Table I of the mothers showing nil swelling, eight had young killed by a dose of toxin of 0·010 to 0·020, nine had young killed by a dose

between 0·008 and 0·010 and four below 0·008, therefore the young of these 21 would all have died if injected with 0·020 and in the same way seven had young capable of surviving an injection of 0·020 c.c.

TABLE II.

Dose at or above which young survived and at or under which young died	Size of local reaction in mother: Nil		Small		Large	
	Survivals	Deaths	Survivals	Deaths	Survivals	Deaths
0·008 c.c.	16	4	6	2	11	0
0·010 c.c.	13	13	6	4	11	0
0·020 c.c.	7	21	3	5	5	1
0·030 c.c.	3	23	2	5	2	1

TABLE III.

Showing the percentage numbers of mothers whose young survived any particular doses.

	Size of local reaction in mother		
Dose	Nil	Small	Large
0·008 c.c.	80	75	100
0·010 c.c.	50	60	100
0·020 c.c.	25	37	83
0·030 c.c.	11	28	66

From Table III it is seen that mixtures nearer L+ than L0 confer higher immunity—the young of over 80 % of the does injected with toxin-antitoxin mixtures producing large local reactions tolerated 0·020 c.c. of toxin, while the young of only 25 % of the does injected with mixtures producing no local reaction tolerated the same dose. It should be pointed out that these tables represent the results from 62 mothers but at least 400 young from these mothers were tested. A somewhat noticeable exception to the figures occurs in the case of two mothers with small local reactions whose young died with doses less than 0·008 c.c. In both cases the pigs were used for testing very weak antitoxic serum and were injected with a test dose of toxin together with ½ c.c. of serum. This quantity of serum in itself is enough to account for a small local reaction on the pig. In both cases the rapid growth of the pigs after the injection indicates that so far as the toxin reaction is concerned the swelling should be considered as nil. Table IV gives the results as they would read if this alteration were made.

1 *b*. Probably the most essential feature of the toxin-antitoxin mixture injected into the mother is the amount of toxin (or toxoid)

dissociated, and this is best indicated by the excess of antitoxin present in the mixture above that necessary to protect life until the 5th day when injected with one test dose of toxin. In many cases the mothers had been used for giving only a rough approximation of the antitoxic

TABLE IV.

Dose	Size of local reaction in mother: Nil	Small	Large
0·008 c.c.	72	100	100
0·010 c.c.	46	75	100
0·020 c.c.	23	50	83
0·030 c.c.	10	40	60

value of a serum and so the exact value of the serum used was not known in all cases. The figures in Tables V, VI and VII give the results for all mothers where it was known that the excess of antitoxin in the mixture was less than 20 %, less than 50 %, or over 50 %.

TABLE V.

Results of immunity of young divided according to the excess of antitoxin present in the toxin-antitoxin mixture injected into the mother.

		Excess of antitoxin: Over 50 %	Under 50 %	Under 20 %
Lowest dose killing young	Under 0·008 c.c. ...	2	1*	0
	0·008 and under 0·010 c.c.	3	1	0
	0·010 ,, 0·020 c.c.	3	2	0
	0·020 ,, 0·030 c.c.	1	0	0
Highest dose tolerated by young	0·008 and under 0·010 c.c.	0	0	0
	0·010 ,, 0·020 c.c.	2†	7	3
	0·020 ,, 0·030 c.c.	0	2	2
	Over 0·030 c.c.	0	3	0

* This pig was paralysed and consequently its responsive power was impaired.

† One of these figures is the result of only a single test upon one young and may be a case of accidental survival through leakage of injection. The other refers to a pig that gave a most unusual result of no local reaction but a marked drop in weight, indicating that the test was "out" in some way.

TABLE VI.

Dose at or above which young survived and at or under which young died	Excess of antitoxin: Over 50 %		Under 50 %		Under 20 %	
	Survivals	Deaths	Survivals	Deaths	Survivals	Deaths
0·008 c.c.	2	2	12	1	5	0
0·010 c.c.	2	5	12	2	5	0
0·020 c.c.	0	8	5	4	2	0
0·030 c.c.	0	9	3	4	0	0

TABLE VII.

Showing the percentage numbers of mothers whose young survived any particular dose.

Dose	Excess of antitoxin		
	Over 50 %	Under 50 %	Under 20 %
0·008 c.c.	50	92	100
0·010 c.c.	28	85	100
0·020 c.c.	0	55	100
0·030 c.c.	0	42	—

Table VII shows very definitely that the immunity conferred by a doe upon the young depends upon the amount in the toxin-antitoxin mixture of toxin (or toxoid) dissociated and so available for immunisation. In the case of eight does where the excess of antitoxin was greater than 50 % none of their young could tolerate 0·020 c.c. of toxin, while the young from five out of nine mothers receiving under 50 % excess of antitoxin tolerated this dose.

1*c*. Another variable in the constitution of the mixture injected into the mother is the constitution of the toxin used. The majority of the results recorded were from mothers injected with the same test toxin but in the few cases where other toxins were used there was a strong indication that a fresh toxin does not give such high immunity as an older toxin where there is more toxoid dissociated in a neutral mixture. The results obtained are too few to form any definite conclusion unless all other variables can be eliminated.

1*d*. Another variable in the constitution of the mixture is the amount of toxin injected. The results recorded so far are from cases where the mother received one test dose of toxin together with antitoxin. In testing the antitoxic value of the serum obtained from apparently normal horses it is often necessary to supply 1/5 or 1/10 of the test dose of toxin. The young bred from mothers used for these tests did not as a general rule give very high immunity. In one case a moderately high degree of immunity was shown, this however was not to be compared with the highest degrees of immunity reached by the young of mothers treated with a full test dose of toxin.

2. *Individual differences in parents.*

In most animals individuals of the same species appear to vary in their responsiveness to immunisation. Various slight irregularities in the foregoing tables are sufficient to show that such variability does exist in guinea-pigs. A definite example is given below.

Two pigs 100 Z and 105 AA were injected with a test dose of the same toxin and 1·3 unit antitoxin. Nine months after the injection the young were tested with 0·015 c.c. toxin, that of 100 Z gave no local reaction, while that of 105 AA gave a very large local reaction and a drop of 35 grams in weight on the fifth day after injection. Subsequent tests of later litters showed that the young of 100 Z would tolerate 0·050 c.c. of toxin better than those of 105 AA would tolerate 0·015 c.c.

3. *Time elapsing between injection of mother and birth of young.*

In the majority of cases the young of the first and possibly the second litter were tested with doses well above or well below the dose they would just tolerate, consequently very few examples illustrate the degree of immunity at all stages in the life of the mother. That a gradual diminution in immunity or at least in the power of transmitting immunity occurs in the mother is shown in the individual cases given in Table VIII.

TABLE VIII.

Cage	Litter	Time elapsing between injection of mother and birth of young	Age of young	Dose injected	Date of death	Local reaction	Change in weight
47 A	1st	13 months	32 days	0·016 c.c.	...	Very large	– 35 grm.
			37 ,,	0·018 ,,	...	,,	– 50 ,,
	2nd	20 ,,	35 ,,	0·006 ,,	...	,,	– 15 ,,
			35 ,,	0·010 ,,	6th day	...	...
105 B	1st	5 ,,	40 ,,	0·022 ,,	...	Very large	– 30 grm.
			44 ,,	0·024 ,,	...	,,	– 55 ,,
			44 ,,	0·026 ,,	...	,,	– 45 ,,
	2nd & 3rd	...	Still borns	...	...	...	...
	4th	16 months	56 days	0·018 c.c.	3rd day	...	...
			56 ,,	0·015 ,,	4th ,,	...	...
153 F	1st	6 ,,	42 ,,	0·012 ,,	...	Very large	+ 10 grm.
			42 ,,	0·024 ,,	...	,,	– 10 ,,
	2nd	...	Still borns	...	...	...	...
	3rd	14 months	35 days	0·028 c.c.	4th day	...	...
			35 ,,	0·025 ,,	5th ,,	...	...
153 AA	1st	9 ,,	18 ,,	0·015 ,,	...	Very large	– 55 grm.
	2nd	12 ,,	32 ,,	0·008 ,,	6th day	...	...
94 B	1st	11 ,,	38 ,,	0·020 ,,	...	Very large	– 5 grm.
	2nd	14 ,,	35 ,,	0·016 ,,	...	,,	– 15 ,,
			42 ,,	0·018 ,,	6th day	...	...
	3rd	20 ,,	50 ,,	0·015 ,,	4th ,,	...	...
			61 ,,	0·012 ,,	5th ,,	...	...
	4th	25 ,,	21 ,,	0·008 ,,	...	Very large	– 20 grm.
			28 ,,	0·008 ,,	...	,,	– 10 ,,

The cases recorded in Table VIII show more or less rapid loss of immunity in the mother, but they cannot be closely analysed in consequence of the variation of immunity according to the age of the young which is to be dealt with next.

We have demonstrated immunity to the extent of tolerating 10 fatal doses of toxin in young born 25 months after the injection of the mother.

4. *The age of the young when tested for immunity.*

In all cases the young are tested when they have reached the weight of 250 grams, the time taken for pigs to reach this weight varies from 10 days to 120 days. Tables IX, X, XI, give the results of over 300 young whose exact ages were known. It should be noted that these tables differ from I, II, III and V, VI, VII as all the young are given irrespective of the treatment of the parents while the earlier tables recorded the mothers and not the young.

TABLE IX.

	Dose tested	Age of young in days: 20 or less	21—30	31—40	41—50	51—60	Over 60
Deaths	Under 0·008 c.c. ...	2	4	2	1	7	14
	0·008 and under 0·010 c.c.	4	21	21	12	4	1
	0·010 ,, 0·020 c.c.	4	12	14	12	13	6
	0·020 ,, 0·030 c.c.	5	7	7	2	9	5
Survivals	0·008 and under 0·010 c.c.	9	10	4	1	1	0
	0·010 ,, 0·020 c.c.	8	17	18	9	3	1
	0·020 ,, 0·030 c.c.	1	7	9	4	5	0
	Over 0·030 c.c. ...	1	3	4	3	0	0

TABLE X.

Doses at or above which young survived and at or under which young died	Age of young in days: 20 or less		21—30		31—40		41—50		51—60		Over 60	
	Survivals	Deaths	Survivals	Deaths	Survivals	Deaths	Survivals	Deaths	Survivals	Deaths	Survivals	Deaths
0·008 c.c.	19	2	37	4	35	2	17	1	9	7	1	14
0·010 c.c.	10	6	27	25	31	23	16	13	8	11	1	15
0·020 c.c.	2	10	10	37	13	37	7	25	5	24	0	21
0·030 c.c.	1	15	3	44	4	44	3	27	0	33	0	26

Table XI shows that immunity has almost disappeared at the end of two months. But that it may occasionally be present, however, to a considerable extent after this period will be shown later when we record cases in connection with another section of the subject.

TABLE XI.

Showing the percentage number of young of different ages that survived any particular doses.

Dose	Age of young in days					
	20 or less	21—30	31—40	41—50	51—60	Over 60
0·008 c.c.	90	90	94	94	56	6
0·010 c.c.	62	51	57	55	47	6
0·020 c.c.	16	21	26	21	17	0
0·030 c.c.	6	6	8	10	0	0

Table XII gives individual cases of the effect of the age of the young.

TABLE XII.

Cage	Time elapsing between injection of mother and birth of young	Age of young	Dose injected	Date of death	Local reaction	Change in weight
47 B	14 months	51 days	0·009 c.c.	3rd day	...	...
		58 ,,	0·007 ,,	5th ,,	...	...
	17 ,,	52 ,,	0·006 ,,	3rd ,,	...	...
		73 ,,	0·0055,,	...	Very large	– 45 grm.
	24 ,,	28 ,,	0·008 ,,	...	,,	– 45 ,,
		32 ,,	0·008 ,,	7th day	...	...
		35 ,,	0·009 ,,	14th ,,	...	...
100 N	9 ,,	50 ,,	0·015 ,,	15th ,,	...	...
	14 ,,	31 ,,	0·020 ,,	...	Very large	– 15 grm.
105	6 ,,	56 ,,	0·030 ,,	7th day	...	...
		70 ,,	0·030 ,,	3rd ,,	...	...
106 D	6 ,,	29 ,,	0·026 ,,	...	Large	+ 25 grm.
	9 ,,	60 ,,	0·020 ,,	...	Very large	– 25 ,,
	14 ,,	36 ,,	0·020 ,,	...	,,	+ 25 ,,
106 L	6 ,,	60 ,,	0·025 ,,	...	Large	– 10 ,,
	11 ,,	29 ,,	0·025 ,,	...	Very small	No change
153 A	5 ,,	50 ,,	0·018 ,,	...	Very large	– 10 grm.
		87 ,,	0·018 ,,	3rd day	...	...

It will be seen from Table XII that in some cases, notably in cage 100 N, young pigs of subsequent litters will tolerate a larger dose than older pigs of earlier litters owing to the more rapid loss of immunity in the young compared to that in the mother. This result is what would have been expected from the fact that passive immunity is more transient than active. The smaller dose tolerated by older pigs is due to loss of antitoxin and not to any weakness of the pig indicated by the longer

time taken to reach the standard weight, because we have shown(8) that for normal guinea-pigs of the same weight the minimal fatal dose increases with age. This is also seen in Table XIII giving the time taken by 0·008 c.c. toxin to kill normal guinea-pigs of different ages during the months of May, June and July, 1909.

TABLE XIII.

	Age of guinea-pigs		
	20—30 days	31—40 days	41—50 days
Time taken to kill	3 days	3 days	4 days
	3 ,,	3 ,,	7 ,,
	3 ,,	4 ,,	11 ,,
	4 ,,	6 ,,	
	4 ,,		

5. *Time of year and climatic conditions.*

This has been shown to have a marked effect on normal guinea-pigs, and a similar effect was noticeable on young showing little or no immunity. The effect has not been demonstrated clearly in the case of young showing considerable immunity. The effect would be quite masked by other variables in any attempt to show the effect by statistical methods.

6. *Individual differences in young.*

Throughout our work there has been evidence that slight individual differences do occur, but great variations in immunity have not been observed. The differences noted were small and mostly to be accounted for by the age of the pigs. It seldom happened that a direct comparison could be made on different individuals of the same litter reaching the standard weight on the same day. In one case however (cage 105 TT) three pigs of the same litter were tested when 35 days old and 250 grams in weight. The dose used was 0·015 c.c. toxin and the results were

Doe ... ... died in three days.
Buck ... ... „ „ seven „
Buck ... ... Survived with very large local reaction and a loss of 50 grams in weight on the fifth day.

General notes on results obtained with young from mothers injected with toxin-antitoxin mixtures.

Results obtained with cage 19 afford a very good series showing the combined influence of the age of young when tested and the time elapsing between injection of mother and birth of young. The figures are given in Table XIV.

TABLE XIV.

Litter	Time elapsing between injection of mother and birth of young	Age of young	Dose injected	Date of death	Local reaction	Change in weight
1, 2	All still born	...	...	...	...	...
3	11 months	49 days	0·018 c.c.	...	Large	-5 grm.
		56 ,,	0·020 ,,	4th day	...	...
4	14 ,,	57 ,,	0·014 ,,	...	Very large	-25 grm.
		62 ,,	0·016 ,,	4th day	...	...
5	19 ,,	70 ,,	0·010 ,,	...	Very large	-10 grm.
		84 ,,	0·012 ,,	5th day	...	...
6	22 ,,	34 ,,	0·012 ,,	...	Large	+15 grm.
		45 ,,	0·015 ,,	...	,,	-15 ,,

In each litter the results are sufficiently clear to enable us to fix a fatal dose as an index of the immunity possessed by the young of that litter.

TABLE XV.

Litter	Time elapsing between injection of mother and birth of young	Average age of young	Minimum fatal dose for litter
3	11	52½ days	0·019 c.c.
4	14	59½ ,,	0·015 ,, −
5	19	77 ,,	0·011 ,, +
6	22	39½ ,,	0·016 ,,

From Table XV it will be seen that the degree of immunity of each litter is in order of the average age of the young of that litter if allowance is made for a slight decrease owing to loss of immunity in mother.

As would be expected the amount of different toxins tolerated by immune young depends not upon the number of fatal doses but upon the binding units. Immune young will tolerate many more fatal doses of a fresh toxin containing little toxoid than of an old toxin rich in toxoids.

The results recorded so far were made with toxin (98 A) of which the average fatal dose for the year could be taken as 0·008 c.c. Another bottle (98 B) of the same toxin was found to have diminished more rapidly in toxicity so that at the time of testing (eight years after the preparation of the toxin) the average fatal dose was 0·014 c.c. It was found that the volumes of 98 A and 98 B tolerated by highly immune young were almost identical. The highest number of fatal doses that immune young survived when tested with 98 A was 7, with another toxin (967 A) the highest number was 14. In volume the highest amounts tolerated were similar and the binding unit content of the two toxins were similar although the fatal dose of 98 A (eight years old) was 0·008 c.c. and that of 967 A (two years old) was 0·004 c.c.

B. *Immunity transmitted to young by mother injected with toxin only.*

It has already been demonstrated clearly by Anderson and Theobald Smith that single injections of sub-lethal doses of toxin in does will not produce immunity in the young. A set of six does was put aside for breeding to confirm this and in no case could we demonstrate immunity in the young. A further set of three does was treated with a series of gradually increasing doses of toxin and again no immunity could be detected in the young. Two of the does treated in this way received similar injections (1/5, 2/5, 2/3 and 1 M.F.D. at weekly intervals). At the end of the treatment one (100 A) had decidedly decreased in weight and the other (100 B) increased. In the case of 100 A only one out of seven young from the first two litters survived while in 100 B four out of six lived. The young of 100 B all showed normal susceptibility to toxin but those of 100 A showed a lowering of resistance as seen in Table XVI. The comparison between the two pigs is shown in Table XVII.

Lustig has shown that immunity to abrin is not transmitted by fowls to their offspring. We shall similarly show in a subsequent section of this investigation that there is evidence against transmission of immunity to diphtheria toxin by the effect on the germ cell before fecundation. Immunity in the young must depend therefore upon the transference of passive immunity from the mother either during pregnancy or by means of the milk. Several experiments were made but only one case was recorded in which a normal pig suckled by a highly immune doe survived the normal lethal dose. In two cases out of three there

appeared to be very slight lowering of the immunity when immune young were suckled by normal does. Thus it appears that immunity is mainly transferred through the placenta during pregnancy. A few experiments were performed to see whether immunity passively acquired by the mother could be so transferred. In eight cases where does were injected at different times during the course of pregnancy with 2000 units of diphtheria antitoxin; no immunity could be detected in the young. This failure may be due to the more rapid loss of passive immunity conferred by the inoculation with antitoxin obtained from another species of animal.

TABLE XVI.

Young from 100 A		Normal young	
Dose	Date of death	Dose	Date of death
0·008 c.c.	5th day	0·009 c.c.	4th day
0·009 ,,	3rd ,,	0·009 ,,	5th ,,
0·009 ,,	3rd ,,	0·009 ,,	5th ,,
		0·009 ,,	6th ,,

TABLE XVII.

Pig	Change in weight during treatment	Resistance of young to Diphtheria toxin	Percentage survivals in first two litters	Percentage survival during total time of breeding
100 A	−20 grm.	Lowered	14	46
100 B	+40 ,,	Normal	66	75

SUMMARY.

1. Diphtheria toxin-antitoxin mixtures induce a higher immunity in guinea-pigs than sub-lethal doses of toxin; one injection of the mixture being sufficient to produce an immunity lasting in some cases for a period of over two years, as shown by the passive immunity conferred on the offspring.

2. The highest immunity is produced by toxin-antitoxin mixtures containing the most uncombined toxoid.

3. The active immunity of the mother is transferred passively to the offspring.

4. The passive immunity thus transferred usually disappears at the end of two months after birth, and only in rare instances has been recognised after three months.

5. Immunity is mainly transmitted in utero, and only to a slight extent during lactation.

6. Young bred from does that have been used for a single routine antitoxin test may be able to tolerate 14 times the dose of diphtheria toxin fatal for a normal guinea-pig.

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IMMUNITY OF GUINEA-PIGS TO DIPHTHERIA TOXIN AND ITS EFFECT UPON THE OFFSPRING.

Part 2.

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C. *Immunity transmitted to young when both parents have been injected with various mixtures of toxin and antitoxin.*

In Part 1 results of the injection of the mothers only have been recorded: we shall now deal with the young of parents which have both received an injection of a toxin-antitoxin mixture. When this work was commenced a limited number of injected pigs were isolated for breeding and their number added to from time to time during the course of these investigations. A complete history was kept of each individual and full notes were taken of all events connected with them from the time of the injection of the parents until the testing of the young of several litters. The number of pigs that could be thus kept apart and individually watched was of necessity strictly limited. In order to obtain easily statistical data that would give indications for subsequent work we kept all survivals from routine toxin-antitoxin injections in runs for breeding without any attempt to isolate individual pigs. The only separation was into two groups—Cage 79 for all pigs that had received injections near the L+ doses, *i.e.* those that had given large local reactions, and Cage 80 for those near the L0 doses, giving little or no reactions. In these runs were put all pigs surviving, whether bucks or does. The general scale of immunity among the young of these pigs was no higher than that among the young from normal bucks and injected does.

This agrees with the generally recognised fact that there is no transmission of immunity through the male.

Figures from some 160 young were obtained from these two groups and results are given here as a confirmation of certain results in Part 1.

Higher Immunity conferred by L+ than by L0 doses.

Higher immunity was obtained in the young from Cage 79 than in those from Cage 80, thus confirming results recorded in Tables I, II, III of Part 1. The figures are given in Tables XVIII, XIX and XX.

TABLE XVIII.

Results of immunity of young divided according to the nature of the toxin-antitoxin mixture injected into the parents.

		Toxin-antitoxin mixture injected into parents	
		L0	L+
Deaths	Under 0·008 c.c.	11	2
	0·008 c.c. and under 0·010 c.c.	20	9
	0·010 ,, ,, 0·020 ,,	12	21
	0·020 ,, ,, 0·030 ,,	6	7
Survivals	0·008 c.c. and under 0·010 c.c.	4	13
	0·010 ,, ,, 0·020 ,,	21	22
	0·020 ,, ,, 0·030 ,,	8	8
	Over 0·030 c.c.	1	1

TABLE XIX.

Dose at or above which young survived and at or under which young died	Toxin-antitoxin mixture injected into parents: L0		L+	
	Survivals	Deaths	Survivals	Deaths
0·008 c.c.	34	11	44	2
0·010 ,,	30	31	31	11
0·020 ,,	9	43	9	32
0·030 ,,	1	49	1	39

TABLE XX.

Showing the percentage number of young surviving any particular dose.

	Toxin-antitoxin mixture injected into parents	
Dose	L0	L+
0·008 c.c.	75	95
0·010 ,,	49	73
0·020 ,,	17	21
0·030 ,,	2	2

As will be apparent, these tables differ in preparation from the corresponding ones in Part 1 since in the one case the mothers were all identified and it was possible to represent their immunity by the highest dose tolerated by, or the lowest dose fatal to, their young. In this way each mother was represented once in the tables. The tables now given are compiled from the degree of immunity shown by the individual young bred in one or other run containing L+ or L0 parents respectively. This difference in the tables is necessary because many of the young could not be identified with a particular mother and in no case was the father known as several bucks were present in each run.

From Table XX it will be seen that 73 % of the young from L+ parents showed slight immunity (*i.e.* survived a dose of 0·010 c.c. toxin) while only 49 % from L0 parents survived the same dose.

Rate of loss of immunity in the young.

Results of tests upon pigs of different ages (but of the same weight —250 grams) were tabulated and are given in Tables XXI, XXII and XXIII, corresponding to Tables IX, X and XI in Part 1.

TABLE XXI.

		Age of young in days					
		20 or less	21—30	31—40	41—50	51—60	Over 60
Deaths	Under 0·008 c.c.	1	1	2	3	4	2
	0·008 c.c. and under 0·010 c.c.	3	9	5	5	2	5
	0·010 ,, ,, 0·020 ,,	0	4	6	9	6	8
	0·020 ,, ,, 0·030 ,,	2	1	4	2	2	2
Survivals	0·008 c.c. and under 0·010 c.c.	4	9	3	1	0	0
	0·010 ,, ,, 0·020 ,,	5	13	10	10	3	2
	0·020 ,, ,, 0·030 ,,	1	7	5	1	0	2
	Over 0·030 c.c.	0	0	0	1	0	1

TABLE XXII.

Doses at or above which young survived and at or under which young died	Age of young in days											
	20 or less		21—30		31—40		41—50		51—60		Over 60	
	Survivals	Deaths	Survivals	Deaths	Survivals	Deaths	Survivals	Deaths	Survivals	Deaths	Survivals	Deaths
0·008 c.c.	10	1	29	1	18	2	13	3	3	4	5	2
0·010 ,,	6	4	20	10	15	7	12	8	3	6	5	7
0·020 ,,	1	4	7	14	5	13	2	17	0	12	3	15
0·030 ,,	0	6	0	15	0	17	1	19	0	14	1	17

From Table XXIII it will be seen that the number of pigs surviving decreases with the increased age after 40 days from birth. The figures are not so regular as in Table XI because the numbers here are fewer. This is particularly the case with the pigs over 60 days old, three exceptional pigs fully account for the irregularity of the figures. These pigs were recorded as three months old and yet showed considerable immunity. These cases are not specially emphasised as we cannot place absolute reliance upon individual results from these groups where the isolation and observation were not as rigid as in the pigs from which Part 1 results were obtained. If we assume that the rate of loss of immunity in the young is the same for the young of all injected does whether the bucks were normal or injected, then we can group the figures from both sets of pigs and obtain statistics from well over 500 pigs. These are given in Tables XXIV, XXV and XXVI. In all cases the pigs were tested directly they reached the weight of 250 grams.

TABLE XXIII.

Showing the percentage number of young of different ages that survived any particular dose.

	Age of young in days					
Dose	20 or less	21—30	31—40	41—50	51—60	Over 60
0·008 c.c.	90	96	90	81	42	71
0·010 ,,	60	66	68	60	33	41
0·020 ,,	20	33	27	10	0	16
0·030 ,,	0	0	0	5	0	5

From Table XXVI it will be seen that up to the age of 40 days at least 90 °/₀ of young survived 0·008 c.c., from 41–50 days 88 °/₀, from 51–60 days 52 °/₀, and over 60 days 27 °/₀. The results in this table appear to

TABLE XXIV.

		Age of young in days					
		20 or less	21—30	31—40	41—50	51—60	Over 60
Deaths	Under 0·008 c.c.	3	5	4	4	11	16
	0·008 c.c. and under 0·010 c.c.	7	30	26	17	6	6
	0·010 ,, ,, 0·020 ,,	4	16	20	21	19	14
	0·020 ,, ,, 0·030 ,,	7	8	11	4	11	7
Survivals	0·008 c.c. and under 0·010 c.c.	13	19	7	2	1	0
	0·010 ,, ,, 0·020 ,,	13	30	28	19	6	3
	0·020 ,, ,, 0·030 ,,	2	14	15	5	5	2
	Over 0·030 c.c.	1	3	4	4	0	1

TABLE XXV.

Doses at or above which young survived and at or under which young died	Age of young in days: 20 or less		21—30		31—40		41—50		51—60		Over 60	
	Sur-vivals	Deaths	Sur-vivals	Deaths	Sur-vivals	Deaths	Sur-vivals	Deaths	Sur-vivals	Deaths	Sur-vivals	Deaths
0·008 c.c.	29	3	66	5	54	4	30	4	12	11	6	16
0·010 ,,	16	10	47	35	47	30	28	21	11	17	6	22
0·020 ,,	3	14	17	51	19	50	9	42	5	36	3	36
0·030 ,,	1	21	3	69	4	61	4	46	0	47	1	43

TABLE XXVI.

Showing the percentage number of young of different ages that survived any particular dose.

Dose	Age of young in days: 20 or less	21—30	31—40	41—50	51—60	Over 60
0·008 c.c.	90	93	92	88	52	27
0·010 ,,	61	57	61	57	39	21
0·020 ,,	17	25	27	17	12	7
0·030 ,,	4	2	6	2	0	2

be almost uniform with any age up to 40 days with the tendency towards a maximum immunity among pigs between 30 and 40 days old rather than among younger pigs. All individual cases recorded indicate definitely that in pigs of the same litter (or in all pigs from the same mother if allowance is made for the progressive loss of immunity in the mother) the youngest pig gives the highest immunity without any such optimum age as would be indicated in Table XXVI. This apparent discrepancy would seem to show that the same causes that tend to yield high immunity in the mother also tend to delay the rate of growth in the subsequent offspring. If this were the case the majority of exceptionally young pigs tested would be the offspring of pigs of less immunity and the first two columns of Table XXVI would refer to pigs of a lower grade of immunity than those of subsequent columns.

D. *Evidence of lowered resistance in young of injected parents.*

While testing young for traces of immunity it was noticed that in some cases the young from injected parents died earlier than the controls. A few isolated instances of earlier deaths would be of no significance because marked differences in the lethal time are fairly frequent in normal pigs injected on the same day. It was noticed, however, that in

the case of certain does a large majority of their young injected with normal lethal doses died earlier than the controls. In such a case one is forced to conclude that either the immunity of the mother has passed off, that the immunity transmitted to the young by the mother has disappeared during the growth of the pig or that the mother failed to produce antitoxin in response to the injection, and that the absence of passive immunity then brings into evidence the damaging action of the toxin-antitoxin injection or of some disturbing factor other than the toxin in the toxin-antitoxin mixture. It is also possible that certain strains of guinea-pigs possess naturally either a greater or less resistance than the normal. This while possibly accounting for certain irregularities, cannot explain such results as are given in Tables XXIX to XXXII.

In Cage 79 where the young were bred from parents that had received L+ doses, only one case was noticed in which the young showed apparently a lowered resistance. This is given in Table XXVII.

TABLE XXVII.

Evidence of lowered resistance among young bred from L+ *parents.*

Cage	Litter	Age of young	Treatment of young: Dose	Treatment of young: Result	Control pig: Dose	Control pig: Result
79 F	1	20 days	0·0085 c.c.	Large reaction increasing 10 grms. in weight	—	—
	2	55 ,,	0·010 ,,	Died in 3 days	0·010 c.c.	Died in 5 days
		62 ,,	0·008 ,,	,, 4 ,,	0·008 ,,	,, 7 ,,
		62 ,,	0·010 ,,	,, 3 ,,	0·010 ,,	,, 3 ,,

A 20-day old pig of the first litter easily survived a normal lethal dose. In the second litter where the pigs were 55 and 62 days old when tested two out of three died earlier than the controls. This result standing alone would be of very little significance but some marked instances occurred in Cage 80 recorded in Table XXVIII. The first litter of pig 80 CC was tested with too high a dose, but in the second litter one of the young died in five days after an injection of 0·005 c.c. when a control normal pig survived a higher dose (0·0055 c.c.). In the third litter all three of the offspring died earlier than the controls. Similar results were obtained with the 2nd, 3rd and 4th litters of 80 N. The greater susceptibility here shown cannot in every case be accounted for by the variation in resistance dependent on the age of the

pig; in 80 CC young of 28 days in one litter and 53 days in another showed lowered resistance while in Cage 80 N young of different litters 14 days and 53 days old died earlier than the controls.

A small summary of Table XXVIII will show that the lowering of resistance is quite definite. In Cage 80 CC four pigs are directly comparable with the controls, of these one died when control survived a higher dose and three died earlier than the normal pigs. In Cage 80 N of five pigs compared two died when controls survived the same dose and the other three died earlier.

It appears advisable to record here other evidence of lowered resistance in young bred from injected does (the case of the offspring of injected bucks and normal does will be dealt with later). Only a few instances were noted among the young of normal bucks and injected does and these are given in Table XXIX.

TABLE XXVIII.

Evidence of lowered resistance among young bred from L0 *parents.*

Cage	Litter	Age of young	Treatment of young: Dose	Treatment of young: Result	Control pig: Dose	Control pig: Result
80 CC	1	49 days	0·016 c.c.	Died in 3 days	0·007 c.c.	Died in 4 days
		49 ,,	0·012 ,,	,, 2 ,,	0·007 ,,	,, 4 ,,
		49 ,,	0·008 ,,	,, 3½ ,,	0·007 ,,	,, 4 ,,
	2	48 ,,	0·0055 ,,	,, 3 ,,	0·006 ,,	,, 3 ,,
		53 ,,	0·0050 ,,	,, 5 ,,	0·0055 ,,	Survived
	3	28 ,,	0·007 ,,	,, 6 ,,	0·007 ,,	Died in 7 days
		28 ,,	0·008 ,,	,, 4 ,,	0·008 ,,	,, 6 ,,
					0·008 ,,	,, 11 ,,
		35 ,,	0·008 ,,	,, 4 ,,	0·008 ,,	,, 7 ,,
					0·008 ,,	,, 7 ,,
80 N	1	21 ,,	0·016 ,,	,, 7 ,,	...	...
		24 ,,	0·017 ,,	,, 3 ,,	...	...
	2	49 ,,	0·008 ,,	,, 4 ,,	0·0065 ,,	Died in 4 days
		49 ,,	0·012 ,,	,, 3 ,,		
		53 ,,	0·007 ,,	,, 4½ ,,	0·007 ,,	Survived
	3	41 ,,	0·0055 ,,	,, 6 ,,	0·0055 ,,	Died in 8 days
		52 ,,	0·006 ,,	,, 5 ,,	0·006 ,,	Survived
	4	14 ,,	0·007 ,,	,, 4 ,,	0·007 ,,	Died in 5 days
		14 ,,	0·008 ,,	,, 4 ,,	0·008 ,,	,, 14 ,,

No instances were noticed among the young from normal bucks and does which had received L+ doses. The cases in Table XXIX were all from L0 does and it is decidedly significant that in each case the injected mixture contained at least ½ c.c. of horse serum. It would

appear that in the usual routine toxin-antitoxin injections, where the quantity of horse serum would average 1/500 c.c., those doses that would affect the mother sufficiently to lower the resistance of the offspring must contain sufficient uncombined toxoid to produce considerable immunity. The degree of lowering of resistance is very small compared with the degree of immunity conferred and so it is rare that lowered resistance can be demonstrated. The effect is presumably non-specific and so could probably be shown if the young were tested

TABLE XXIX.

Evidence of lowered resistance among young bred from normal bucks, and does which had been injected with toxin-antitoxin mixtures.

			Treatment of young		Control pig	
Cage	Litter	Age of young	Dose	Result	Dose	Result
106 P	1	45 days	0·015 c.c.	Died in 2 days	0·0065 c.c.	Died in 5 days
	2	66 ,,	0·015 ,,	,, 2 ,,	0·006 ,,	,, 3 ,,
		80 ,,	0·006 ,,	,, 2 ,,	0·006 ,,	,, 5 ,,
		82 ,,	0·0055 ,,	,, 3 ,,	0·0055 ,,	Survived
					0·006 ,,	Died in 5 days
127 L	1	58 ,,	0·015 ,,	,, 3 ,,	0·006 ,,	,, 5 ,,
		66 ,,	0·006 ,,	,, 5 ,,	0·006 ,,	Survived
					0·0065 ,,	Died in 8 days
	2	28 ,,	0·008 ,,	,, 3 ,,	0·008 ,,	,, 5 ,,
		28 ,,	0·008 ,,	,, 4 ,,	0·008 ,,	,, 5 ,,
		31 ,,	0·008 ,,	,, 5 ,,	0·008 ,,	,, 5 ,,
					0·008 ,,	,, 11 ,,
105 BB	1	25 ,,	0·012 ,,	,, 3 ,,	0·006 ,,	Survived
	2	35 ,,	0·008 ,,	,, 3 ,,	0·008 ,,	Died in 5 days
		35 ,,	0·008 ,,	,, 4 ,,	0·008 ,,	,, 5 ,,
		52 ,,	0·008 ,,	,, 3 ,,	0·008 ,,	,, 4 ,,

against a toxin other than that to which they are passively immune. A few pigs bred from mothers highly immune to diphtheria toxin were tested against living typhoid organisms but no reliable results were obtained owing to the great range of inaccuracy. It follows that, in dealing with young bred from normal bucks and injected does, lowered resistance can only be demonstrated in those cases where the effect on the general vitality of the mother is due to causes other than a simple toxin-antitoxin mixture, such as $\frac{1}{2}$ c.c. of horse serum as in the cases under consideration. It is possible that the cases in Tables XXVII and XXVIII were of does which had received injections containing large quantities of horse serum. It should again be stated that Tables XXVII

and XXVIII refer to pigs from cages 79 and 80 where the individual treatment was unknown.

In the course of experiments as to whether passive immunity in the mother could be transmitted to the young, various does were injected with 5 c.c. (5000 units) diphtheria antitoxic serum, before and during pregnancy, and again immediately after birth. The number of young surviving was not large and the results of all those tested for one lethal dose are recorded in Tables XXX, XXXI, XXXII.

TABLE XXX.

Evidence of lowered resistance among young bred from normal bucks, and does which had been injected with antitoxic serum alone.

			Treatment of young		Control pig	
Cage	Litter	Age of young	Dose	Result	Dose	Result
133	1	73 days	0·007 c.c.	Died in 7 days	0·007 c.c.	Survived
151	1	Died before reaching standard weight				
	2	69 days	0·006 c.c.	Survived, lost 65 grms.	0·006 ,,	Survived, lost 5 grms.
	3	43 ,,	0·008 ,,	Died in 4 days	0·008 ,,	Died in 4 days
		50 ,,	0·008 ,,	,, 4 ,,	0·008 ,,	,, 5 ,,
					0·008 ,,	,, 5 ,,

TABLE XXXI.

Evidence of lowered resistance among young from normal does injected with antitoxic serum during pregnancy.

			Treatment of young		Control pig	
Cage	Litter	Age of young	Dose	Result	Dose	Result
78	1	36 days	0·0085 c.c.	Died in 5 days	...	...
		59 ,,	0·013 ,,	,, 3 ,,	0·013 c.c.	Died in 3 days
120	1	44 ,,	0·014 ,,	,, 3 ,,		
		44 ,,	0·014 ,,	,, 5 ,,	0·014 ,,	,, 5 ,,
		44 ,,	0·015 ,,	,, 4 ,,		
121	1	22 ,,	0·014 ,,	,, 4 ,,	...	...
		22 ,,	0·016 ,,	,, 4 ,,	...	...
		23 ,,	0·014 ,,	,, 3 ,,	0·014 c.c.	Died in 8 days

In each table the young appear slightly less resistant than normal pigs. In all cases the conditions of breeding and growth of the young were the same as those for the normal control pigs except the injection of serum into the mother. If the resistance of these pigs was quite

TABLE XXXII.

Evidence of lowered resistance among young suckled by normal mothers injected with antitoxic serum immediately after birth of young.

			Treatment of young		Control pig	
Cage	Litter	Age of young	Dose	Result	Dose	Result
82	1	39 days	0·008 c.c.	Died in 2 days	0·008 c.c.	Died in 5 days
84	1	18 ,,	0·008 ,,	,, 3 ,,	0·008 ,,	,, 8 ,,
		20 ,,	0·008 ,,	,, 3 ,,	...	...
122	1	22 ,,	0 014 ,,	,, 3 ,,	0·013 c.c. 0 015 ,,	Died in 3 days ,, 3 ,,
		25 ,,	0·013 ,,	,, 7 ,,	0·013 ,,	Survived
123	1	24 ,,	0·014 ,,	,, 3 ,,	0·014 ,,	Died in 3 days
		35 ,,	0·014 ,,	,, 3 ,,	0·016 ,,	,, 3 ,,

normal we could expect that the majority would die in the same time as controls injected with the same dose, a few would die earlier, a few later. A summary of the results from the three tables shows that 14 young from injected mothers can be compared directly with normal control animals, that none died later than the controls, six died in the same time, five died earlier, two died when the control lived and one survived but lost 65 grams in weight where the control lost only 5 grams. Thus from 14 young from injected mothers, eight showed lowered resistance. It would appear that in each case where the vitality of the mother was slightly affected by the injection of a foreign protein the resistance of the young to diphtheria toxin was lowered to a slight extent.

E. *Active immunity conferred by single injections of toxin in pigs that have been rendered passively immune by maternal transmission.*

That the immunity conferred on the young of immune does is not transmitted to the next generation has been shown by all investigators who have taken up the subject of the transmission of immunity from parents to their offspring. This is in accord with the fact that the immunity so conferred is of a passive kind and is lost well before the age at which pigs commence to breed. Our own experiments were performed with five first-generation pigs from mothers whose other offspring had shown good immunity. These pigs were paired with normal bucks and the young tested as soon as they had attained the weight of 250

grams; neither immunity nor increased susceptibility was shown, all died within a short time of the controls, three slightly earlier, three at the same time and three slightly later.

We have already seen in Part 1 Section B that sublethal doses of toxin (without antitoxin) injected into normal pigs produce no appreciable immunity in their young. The following results show that a single injection of toxin into a doe rendered passively immune by maternal transmission may result in the young of such an animal exhibiting a considerable degree of immunity.

An example may be recorded of a doe in Cage $79S_2$ bred from parents that had both received L+ doses. This pig was born 19. IV. '08 and on 1. VI. '08 when 250 grams in weight was injected with 0·0095 c.c. toxin, there was no local reaction and no change in weight. A control normal pig injected with the same dose on the same day died in seven days. The doe was paired with a normal buck on 29. VII. '08 and gave birth to two young on 4. XII. '08, to still-borns on 15. III. '09 and 21. V. '09, and to three young again on 6. VIII. '09. The results of the tests upon the young were as follows.

Born 4. XII. '08.	Tested when 33 days old.	0·0055 c.c.	Large reaction and increased 20 grms. in weight.
,, ,,	,, ,, ,,	0·010 ,,	Large reaction and lost 10 grms. in weight.
	Control pig	0·0055 ,,	Died in 7 days.
Born 6. VIII. '09.	Tested when 25 days old.	0·008 ,,	Very large reaction, increased 25 grms. in weight.
,, ,,	,, ,, ,,	0·015 ,,	Very large reaction, lost 25 grms. in weight.
,, ,,	,, ,, ,,	0·030 ,,	Died in 4 days.
	Control pig	0·007 ,,	,, 5 ,,

In this case an approximate normal lethal dose only was injected into the immune pig of the first generation, with the result that considerable immunity was conferred on the young of the second generation.

Contrasted with this case is that of a doe in Cage 79 P_1, also bred from parents that had both received L+ doses. This pig was born 1. V. '08 and on 4. VI. '08 when 250 grams in weight was injected with 0·012 c.c. toxin. There was a large local reaction and a loss of 40 grams in weight. A control normal pig was injected with 0·011 c.c. toxin and died in 9 days. The doe was paired with a normal buck on 8. I. '09 and young were born 25. V. '09 and 17. VIII. '09. The results of the tests upon the young were as follows.

Born 25. v. '09.	Tested when 14 days old.	0·008 c.c.		Very large reaction, lost 30 grms.
,, ,,	,, ,, ,,	,,		,, ,, ,, 40 ,,
	2 Controls	,,		Both died in 5 days.

Born 17. viii. '09.	Tested when 45 days old.	0·010 c.c.	98 B[1].	Very large reaction, lost 65 grms.
,, ,,	,, ,, ,,	0·016 ,,	,,	Died in 3 days.
,, ,,	,, ,, ,,	0·020 ,,	,,	,, 3 ,,
	Control	0·016 ,,	,,	,, 7 ,,
	,,	0·020 ,,	,,	,, 4 ,,

In this case second generation young only 14 days old showed very slight immunity, while older pigs of the next litter showed distinct evidence of lowered resistance.

The main difference between the two cases was the degree to which the mother was affected by the dose of toxin. Little or no immunity was transmitted when the mother was severely affected, while there was considerable immunity in the young whose mother easily tolerated the dose of toxin given. Similar results were noted in many instances and these are recorded in detail in Tables XXXIII, XXXIV and XXXV, divided according to the degree to which the mother was affected.

In Table XXXIII are recorded results of tests upon the young of seven immune does, all of which easily survived the dose given. In all cases the young showed decided immunity. As much as 10 fatal doses of toxin jar 967 A was tolerated by the young of 164 L_2.

Table XXXIV gives the results for eight does moderately affected by the toxin injected. Two cases, 164 S and 153 S, show little or no immunity.

The size of the local reaction and the change in weight are only general indications of the degree to which a pig is affected by an injection. The changes in weight recorded are those that take place in five days after the injection, a big decrease in weight (25 grams or more) or an increase are significant, but the

[1] The supply of test toxin 98 A, with which all the earlier tests were carried out, ran short in August 1909, and toxin 98 B, and in a few instances 967 A, was then used. In all results given so far no toxin number has been recorded because 98 A was used throughout, in all subsequent tables the toxin number is given. The relative values of the toxins used are as follows:

Original M.F.D. 98 A and B under 0·003 c.c.	Average M.F.D. when used 98 A, 0·008 c.c. ,, ,, ,, ,, 98 B, 0·014 ,,
Original M.F.D. 967 A 0·002 c.c.	,, ,, ,, ,, 967 A, 0·004 ,,

98 A and B were two bottles filled at the same time with the same toxin prepared in 1900, 967 A was prepared in March 1909. The L0 doses were similar, that of 98 A 0·27 c.c. and that of 967 A 0·32 c.c.

TABLE XXXIII.

Results of second generation tests when immune mothers easily survived the toxin injected.

Cage	Injection of mother: Dose (c.c.)	Injection of mother: Reaction	Injection of mother: Change of weight	Time elapsing between injection of mother and birth of young	Age of young	Injection of young: Dose (c.c.)	Injection of young: Toxin	Injection of young: Result[1]	Control pig: Dose (c.c.)	Control pig: Result
100 J	0·010	Nil	+10 grms.	7 months	30 days	0·012	98 A	Nil+25	0·0055	Died in 5 days
					59 ,,	0·020	,,	Died in 7 days	0·0060	,, 6 ,,
				13 ,,	10 ,,	0·016	,,	Large −10	0·0070	,, 6 ,,
79 S_2	0·0095	Nil	No change	6 ,,	33 ,,	0·0055	,,	Large+20	0·0055	,, 7 ,,
						0·010	,,	,, −10		
			–	14 ,,	25 ,,	0·0080	,,	Very large+25	0·0070	,, 5 ,,
					25 ,,	0·015	,,	,, ,, −25		
					25 ,,	0·030	,,	Died in 4 days		
127 BB	0·009	Trace	+25 grms.	10 ,,	19 ,,	0·016	98 B	Very large+15	0·016	,, 7 ,,
94 NN	0·016	Large+20	+20 ,,	3 ,,	31 ,,	0·020	,,	Trace+5	0·016	,, 7 ,,
				6 ,,	21 ,,	0·030	,,	Very large+10	0·016	,, 7 ,,
94 BB	0·016	Very large	+45 ,,	11 ,,	14 ,,	0·040	,,	,, −5	...	...
	0·020[2]	,,	+40 ,,							
164 L_2	0·024	,,	+40 ,,	9 ,,	55 ,,	0·030	,,	,, −5	...	...
				13 ,,	35 ,,	0·030	,,	,, −15	0·020	Died in 7 days
					35 ,,	0·050	,,	,, −55		
					35 ,,	0·040	967 A	,, −5	0·004	,, 5 ,,
94 C	0·020	Large	+10 ,,	5 ,,	35 ,,	0·006	98 A	Nil+15	0·006	,, 4½ ,,
					35 ,,	0·010	,,	Trace+5		
				9 ,,	21 ,,	0·009	,,	Medium+20	0·009	,, 5 ,,
									0·009	,, 6 ,,
				12 ,,	31 ,,	0·009	,,	Medium+30	0·008	,, 4 ,,
					31 ,,	0·009	,,	Large+15		
					31 ,,	0·018	,,	Small+10		

[1] In this and subsequent tables under column headed "Result" is given size of local reaction and number of grams gained or lost in weight during 5 days after the injection.

[2] A second injection given 10 days after the first.

TABLE XXXIV.

Results of second generation tests when immune mothers were moderately affected by toxin injected.

	Injection of mother			Time elapsing between injection of mother and birth of young		Injection of young			Control pig	
Cage	Dose (c.c.)	Reaction	Change of weight		Age of young	Dose (c.c.)	Toxin	Result	Dose (c.c.)	Result
164 L_4	0·040	Very large	No change	6 months	34 days	0·020	98 B	Large + 20	0·016	Died in 7 days
				9 ,,	39 ,,	0·030	,,	Very large + 20	...	...
				12 ,,	—	0·030	,,	,, − 15	0·016	Died in 3 days
79 B_1	0·010	Small	− 25	11 ,,	25 ,,	0·008	98 A	,, + 25	0·007	,, 6 ,,
				16 ,,	41 ,,	0·020	98 B	Died in 6 days	0·014	,, 7 ,,
					48 ,,	0·020	,,	Very large − 15	0·016	,, 19 ,,
					52 ,,	0·020	,,	Died in 2 days	0·016	,, 7 ,,
					63 ,,	0·008	967 A	,, 2 ,,	0·0035	,, 4 ,,
19 F	0·010	Very large	− 10	7 ,,	46 ,,	0·016	98 B	Very large + 10	0·016	Very large − 50
					49 ,,	0·020	,,	,, − 15	0·016	Died in 7 days
					60 ,,	0·008	967 A	Died in 3 days	0·0035	,, 4 ,,
79 AR	0·015	Large	− 5	7 ,,	32 ,,	0·020	98 B	Very large + 20	0·014	,, 7 ,,
				14 ,,	21 ,,	0·040	,,	,, − 5	0·018	,, 7 ,,
79 B_2	0·016	Very large	− 15	13 ,,	28 ,,	0·016	98 A	,, − 25	0·008	,, 5 ,,
					32 ,,	0·030	,,	Died in 4 days	0·008	,, 5 ,,
				18 ,,	28 ,,	0·020	98 B	Very large − 25	0·016	,, 7 ,,
				20 ,,	32 ,,	0·016	,,	,, + 30	...	...
164 S	0·022	Large	− 5	11 ,,	17 ,,	0·016	98 A	Died in 3 days	0·0080	Died in 4 days
					24 ,,	0·0080	,,	Very large − 15	0·0080	,, 5 ,,
158 D	0·022	,,	− 5	8 ,,	21 ,,	0·0080	,,	Nil + 10	0·0080	,, 3 ,,
153 S	0·024	,,	No change	11 ,,	38 ,,	0·015	,,	Died in 3 days	0·0070	,, 5 ,,
					52 ,,	0·0080	,,	,, 5 ,,	0·0080	,, 5 ,,
					57 ,,	0·0080	98 B	Very large − 50	...	...

Results of second generation tests when immune mothers were markedly affected by toxin injected.

Cage	Injection of mother: Dose (c.c.)	Injection of mother: Reaction	Change of weight	Time elapsing between injection of mother and birth of young	Age of young	Injection of young: Dose (c.c.)	Injection of young: Toxin	Injection of young: Result	Control pig: Dose (c.c.)	Control pig: Result
79 P_1	0·012	Large	– 40	11 months	14 days	0·008	98 A	Very large – 30	0·008	Died in 5 days
					14 ,,	0·008	,,	,, – 40	0·008	,, 5 ,,
				14 ,,	45 ,,	0·010	98 B	,, – 65	0·016	,, 7 ,,
					45 ,,	0·016	,,	Died in 3 days	0·016	,, 4 ,,
					45 ,,	0·020	,,	,, 3 ,,		
106 PP	0·018	Very large	– 50	13 ,,	21 ,,	0·008	98 A	,, 5 ,,	0·008	,, 4 ,,
					25 ,,	0·008	,,	,, 5 ,,	...	...
132 R	0·020	,, ,,	– 35	7 ,,	23 ,,	0·009	,,	,, 3 ,,	0·009	Died in 5 days
									0·009	,, 7 ,,
				10 ,,	17 ,,	0·008	,,	,, 5 ,,	0·008	,, 5 ,,
									0·008	,, 11 ,,
46 A	0·020	,, ,,	– 20	9 ,,	28 ,,	0·008	,,	,, 3 ,,	0·008	,, 6 ,,
									0·008	Very large + 15
					38 ,,	0·008	,,	,, 5 ,,	0·008	Died in 4 days
									0·008	,, 5½ ,,
					42 ,,	0·008	,,	,, 4 ,,	0·008	,, 3 ,,
									0·008	,, 5 ,,
127 LL	0·020	,, ,,	– 45	11 ,,	52 ,,	0·020	,,	,, 3 ,,	0·070	,, 5 ,,
					56 ,,	0·008	,,	,, 6 ,,	0·0080	,, 5 ,,
					56 ,,	0·007	,,	,, 7 ,,	0·0070	,, 5 ,,
79 PP	0·020	,, ,,	– 25	6 ,,	42 ,,	0·008	,,	,, 4 ,,	0·008	,, 5 ,,
									0·008	,, 7 ,,
					45 ,,	0·008	,,	,, 4 ,,	0·009	,, 5 ,,
									0·009	,, 6 ,,
				10 ,,	18 ,,	0·007	,,	,, 6 ,,	0·007	,, 4 ,,
					21 ,,	0·008	,,	,, 5 ,,	0·008	,, 7 ,,
									0·008	,, 7 ,,
				15 ,,	56 ,,	0·012	98 B	,, 6 ,,	0·016	,, 5 ,,
					56 ,,	0·016	,,	,, 4 ,,		
153 Z	0·026	,, ,,	– 20	7 ,,	21 ,,	0·0090	98 A	Very large + 5	0·0090	,, 3 ,,
80 X	0·030	,, ,,	– 55	21 ,,	11 ,,	0·018	98 B	,, 5	0·018	Very large – 65

meaning of a small decrease is not clear unless the pig is kept under observation for a considerable time until it regains its normal rate of growth. A small decrease (5 or 10 grams) would indicate a severe reaction if the general conditions of time of year, feeding, etc. were conducive to rapid growth, and if the weight of the pig did not rise considerably for some time. On the other hand, if conditions are unfavourable for rapid growth, as in the winter time, a small decrease in weight in five days, followed immediately by a normal rise, indicates very little constitutional disturbance. Thus it follows that Table XXXIV may include pigs affected with markedly different degrees of severity and that if these pigs had been kept under close observation for a longer time after tne injection, some would have been included in Table XXXIII and some in Table XXXV. The main comparison, therefore, must be made between Tables XXXIII and XXXV.

In Table XXXV results are given for the young of eight does, all of which were severely affected. Three only of these produced young which survived a normal lethal dose. These cases are as follows.

Cage 79 P_1, 14 days old young of first litter survived, older pigs of a subsequent litter showed no immunity.

Cage 153 Z. Only one test made on a 21 day old pig.

Cage 80 X. Only one test made on an 11 day old pig.

The young from the other five does showed no trace of immunity whatever, on the contrary there was evidence of an increased susceptibility as shown in Cage 79 PP, where five out of six young died earlier than the controls injected with the same dose.

From the three cases where very young offspring survived the normal lethal dose it would appear that pigs of this group may transmit a trace of immunity to their young. This trace is rapidly lost and any effect on the vitality of the offspring due to the severity of the reaction caused by the injection of the parent is then exhibited by a condition of greater susceptibility to the injection of toxin.

From these results it would appear that immunity in the second generation depends considerably, if not entirely, upon the ease with which the first generation doe tolerated the toxin injected. This accords with the experience gained in the course of general work on immunisation in which it is found that the most effective response to the production of antibodies is obtained when the dose administered produces a moderate disturbance only; if this is much exceeded so that the animal is severely affected its capacity for producing antibodies in response to the injection is greatly impaired.

F. Immunity in young of the third generation.

The results of the preceding section indicate a possibility of producing very high immunity in young whose female ancestors have received suitable stimuli in each generation. It would also seem possible to produce young of very low resistance in a similar way. Experiments have been commenced with these ends in view and the results thus far obtained are recorded in Tables XXXVI, XXXVII, XXXVIII and XXXIX, where the history of each generation is given. The two second generation pigs surviving in Cage 79 P_1 recorded in Table XXXV were each paired with a normal buck and their young showed indications of lowered resistance. These are detailed in Tables XXXVI and XXXVII. The two other cases of third generation pigs given in Tables XXXVIII and XXXIX were those whose parents and grandparents easily survived the doses given (see Table XXXIII, Cage 94 C and Table XXXIV, Cage 158 D). These pigs showed considerable immunity, particularly in the case of Table XXXIX, where a pig 47 days old gained in weight after an injection of 0·060 c.c. jar 98 B. This dose represents nearly a $\frac{1}{4}$ of the L0 dose, *i.e.* 50 binding units of toxin.

At the time of going to press another third generation result has been obtained confirming those of Tables XXXVIII and XXXIX. Two pigs in cage 223 LA survived and gained in weight after injections of 6 and 10 fatal doses of a fresh toxin. The male parents were normal in each generation and of the females the great-grandmother was injected with a toxin-antitoxin mixture near to the L+ dose causing a large local reaction and loss in weight. The grandmother survived 0·024 c.c. of toxin 98 A with a large increase in weight, the mother survived 0·030 c.c. 98 B with a slight loss in weight.

TABLE XXXVI.

Doe injected with an L+ mixture during 1907.
Paired with a similarly injected buck.

↓

Doe born 1. v. '08, injected 4. vi. '08 with 0·012 c.c. 98 A. Large reaction, lost 40 grms. in weight. (Control injected with 0·011 c.c., died in 9 days.)
Paired with a normal buck.

↓

Doe born 25. v. '09, injected 8. vi. '09 with 0·008 c.c. 98 A. Very large reaction, lost 30 grms. in weight. (Control injected with 0·008 c.c., died in 5 days.)
Paired with a normal buck.

↓

Born 21. xii. '09, injected when 45 days old with 0·016 c.c. 98 B, died in 4 days. (Control injected with 0·016 c.c., died in 5 days.)

TABLE XXXVII.

Doe injected with an L+ mixture during 1907.
Paired with a similarly injected buck.

↓

Doe born 1. v. '08, injected with 0·012 c.c. 98 A. Large reaction, lost 40 grms. in weight. (Control injected with 0·011 c.c., died in 9 days.)
Paired with a normal buck.

↓

Doe born 25. v. '09, injected with 0·008 c.c. 98 A. Very large reaction, lost 40 grms. in weight. (Control injected with 0·008 c.c., died in 5 days.)
Paired with a normal buck.

↓

Born 18. i. '10. Injected when 31 days old with 0·012 c.c. 98 B. Very large reaction, lost 45 grms. in weight. (Control injected with 0·014 c.c., lost 45 grms.)

Born 3. v. '10. Injected when 21 days old with 0·016 c.c. 98 B. Very large reaction, lost 80 grms. in weight. (Control injected with 0·018 c.c., lost 75 grms.)

Born 12. viii. '10. Injected when 25 days old with 0·012 c.c. 98 B. Died in 8 days.
,, ,, ,, ,, 25 ,, 0·018 ,, ,, ,, 4 ,,
(Control injected with 0·018 c.c., died in 7 days.)

TABLE XXXVIII.

Doe injected with L0 dose 22. I. '08.
Paired with a normal buck.

↓

Doe born 29. V. '08, injected 6. VII. '08 with 0·020 c.c. 98 A. Large reaction and increase of 10 grms. in weight. (Control injected with 0·016 c.c., died in 3 days.)
Paired with a normal buck.

↓

Doe born 20. VII. '09, injected 20. VIII. '09 with 0·010 c.c. 98 A. Small reaction and increase of 10 grms. in weight.
Paired with a normal buck.

↓

Born 8. III. '10, injected when 35 days old with 0·030 c.c. 98 B. Very large reaction. No change in weight. (Control injected with 0·016 c.c., died in 7 days.)

Born 1. VIII. '10, injected when 32 days old with 0·050 c.c. 98 B. Very large reaction. Lost 10 grms. in weight. (Control injected with 0·016 c.c., died in 3 days.)

Born 1. VIII. '10, injected when 32 days old with 0·030 c.c. 98 B. Large reaction. Gained 15 grms. in weight. (Control injected with 0·016 c.c., died in 3 days.)

TABLE XXXIX.

Doe injected with an L0 mixture 15. II. '08.
Paired with a normal buck.

↓

Doe born 28. VIII. '08. Injected 1. IX. '08 with 0·022 c.c. 98 A. Large reaction. Lost 5 grms. in weight. (Control injected with 0·012 c.c., died in 3 days.)
Paired with a normal buck.

↓

Doe born 21. V. '09. Injected 11. VI. '09 with 0·008 c.c. 98 A. No reaction. Gained 10 grms. in weight. (Controls injected with 0·008 c.c., died in 3 and 4 days.)
Paired with a normal buck.

↓

Born 12. V. '10. Injected when 43 days old with 0·040 c.c. 98 B. Medium reaction. Gained 10 grms. (Control injected with 0·018 c.c., died in 6 days.)

Born 12. V. '10. Injected when 47 days old with 0·060 c.c. 98 B. Very large reaction. Gained 5 grms. (Control injected with 0·018 c.c., died in 7 days.)

Born 6. IX. '10. Injected when 24 days old with 0·040 c.c. 98 B. No reaction. Gained 30 grms. (Control injected with 0·020 c.c., died in 4 days.)

Born 6. IX. 10. Injected when 24 days old with 0·060 c.c., no reaction. Gained 40 grms. (Control injected with 0·020 c.c., died in 4 days.)

Summary.

(1) The young of parents both of which have been injected with an immunising mixture of diphtheria toxin and antitoxin, show immunity of the same order as that of young from similarly treated mothers and normal fathers.

(2) The injection of certain foreign substances into a female guinea-pig appears to have a direct effect on the offspring in diminishing their resistance to diphtheria toxin, shown equally well by the young of mothers injected, (*a*) before the attainment of sexual maturity, (*b*) during pregnancy, and (*c*) after birth during the period of lactation.

(3) A single injection of diphtheria toxin may give rise to a condition of active immunity (as tested by the resistance of the young) in guinea-pigs possessing hereditarily transmitted passive immunity. Should this injection of toxin give rise to great constitutional disturbance, the young may show lowered resistance, whereas, if it give rise to but slight constitutional disturbance, the young show a high degree of immunity. These effects appear to be accentuated if similar injections are repeated in the next generation.

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IMMUNITY OF GUINEA-PIGS TO DIPHTHERIA TOXIN AND ITS EFFECT UPON THE OFFSPRING.

Part 3.

(*Continued from Vol. XI, p.* 442.)

By H. J. SÜDMERSEN, Ph.D.
and A. T. GLENNY, B.Sc.

G. *Evidence of lowered resistance in the offspring of normal does and injected bucks.*

The results of our tests upon the young of normal does and immune bucks agree with those of other workers in showing that no immunity is transmitted through the male parent. At first only a few experiments were performed to act as a confirmation of work already done by those previously engaged upon the subject, but in these few experiments not only was no immunity shown, but there appeared slight evidence that the young of normal mothers and injected fathers possessed increased susceptibility to diphtheria toxin. Such evidence was also seen in the case of the young of normal fathers and injected mothers as already recorded in Part 2. It appeared, therefore, of importance to endeavour to increase the number of observations until the lowering of resistance of offspring from an injected male parent could be either definitely established or disproved. It was found that the number of experiments necessary to establish the point firmly was prohibitive, but a sufficient number of observations were made to strengthen considerably the probability of the correctness of our conclusions. Evidence submitted by other workers may be forthcoming that taken together with our own results may form conclusive evidence.

When a number of normal guinea-pigs of the same weight and age are injected with the same volume of the same toxin and kept under identical conditions the majority of animals will die at the same time, but some

marked discrepancies will occur. The dose of toxin used as the Normal Lethal Dose in these experiments was that which usually caused the death of a guinea-pig on the fifth day after the injection. Many normal guinea-pigs injected with this dose would die earlier (mostly on the 4th day) while in others, death would be delayed until the 6th to 10th day, while an occasional pig might even survive.

As has already been pointed out, the quantity of a given toxin that will kill a guinea-pig within a certain time varies with the time of year, and so in all cases normal pigs were injected as controls to the experimental animals and the dose was varied from time to time as was necessary to make allowance for the slight seasonal variation in order to keep the average lethal time as near to five days as possible.

The difference noted in the young of injected bucks was of the same order as the difference occurring among normal pigs and very few individual cases were sufficiently marked to be of any significance, but it was repeatedly seen that the difference between the groups of pigs was always in the same direction. An analysis of results showed that when pigs bred from injected bucks and normal does were injected at the same time as normal control pigs of the same weight with the same dose of toxin, at least half of the young from injected bucks died earlier than the young from normal parents. The results are summarised according to whether the experimental young died when the controls survived, or whether they died earlier, at the same time (within 12 hours), or later, or whether they lived when the controls were killed by the same dose. If the pigs under consideration were of normal susceptibility it would be expected that the numbers dying earlier or later than the mean time of death of the controls would balance. To show how far this would be tiue among relatively small groups of pigs the results of all pairs of normal pigs treated upon the same day with the same dose of toxin have been analysed in Table XL. An arbitrary distinction between the two pigs in each instance was made by comparing the result of the pig marked with the earlier letter with that of the other which thus takes the place of 'control.' This distinction can have no bearing upon the results because in all cases the normal pigs to be injected are collected together and then taken up indiscriminately and marked with a distinctive letter; later, at the time of injection the marked pigs are taken one at a time without any regard to the letters by which they are known and not until each pig is injected is its distinctive letter noted. Thus it will be seen that the division made is quite arbitrary and the summary of results should give a close

approximation of what would be expected between our experimental young and the normal controls if the former were of normal susceptibility to diphtheria toxin. Table XL gives two groups of pigs—those injected with Toxin jar 98 A with which all the tests recorded in this section were performed, and those injected during the same period with any other toxin that was tested on pairs of normal pigs. In both groups the figures are closely balanced—in the first group 13 appear more susceptible and 12 less so, and in the other group the corresponding numbers are 24 and 26.

TABLE XL.

Comparing the results of the injection of the same dose of toxin at the same time into pairs of normal pigs divided according to an arbitrary distinction between the members of each pair.

	Number of pigs marked with the earlier letter				
Toxin tested	Dying when other lived	Dying earlier	Dying at the same time	Dying later	Living when other died
Jar 98 A	1	12	13	11	1
Other toxins	2	22	21	23	3
Total	3	34	34	34	4

These figures are interesting in showing the extent of variation to be expected when testing the Minimal Lethal Dose of diphtheria toxin upon guinea-pigs, but further analysis of or comments upon these figures is beyond the range of the present paper. We might, however, note in passing that, from this table, 7 survivals may be expected out of a total of 218 pigs injected with a dose that usually kills on the fifth day.

The total number of experimental young (*i.e.* young got from normal does by injected bucks) tested was 107 and of these 8 died where control survived as opposed to 1 living whose control died and 48 died earlier as compared with 20 dying later. The total number of results given in Table XL is practically the same and these are contrasted in Table XLI.

TABLE XLI.

Contrasting the results of injection of toxin into experimental young (i.e. young got from normal does by injected bucks) compared with normals and results obtained on comparing pairs of normal pigs.

	Number of pigs under observation					
	Dying when control lived	Dying earlier	Dying at same time	Dying later	Living when other died	Total
Normal pigs	3	34	34	34	4	109
Experimental young	8	48	30	20	1	107

A marked contrast will be seen in the results of the experimental young as compared with the normal, shown by a shifting of the figures to the left indicating increased susceptibility.

We have now to consider to what extent these figures are significant. It is possible that the conditions under which the experimental young were bred differed sufficiently from the normal to account for these differences. Normal pigs were bred in large open runs from which does were removed to single cages when well advanced in pregnancy, while the majority of experimental young were bred in single cages containing the injected buck and a normal doe. A total of 41 experimental young were bred under conditions identical with those for the normal controls and of these 21 appeared more as compared with 10 less susceptible than the controls. These figures are given in Table XLII contrasted with a batch of normal pairs of similar numbers.

TABLE XLII.

Contrasting the results of injection of toxin into experimental young compared with normals and the results obtained in comparing pairs of normal pigs, taking into account only such experimental pigs as were bred under conditions identical with those of the normal.

	Number of pigs under observation					
	Dying when control lived	Dying earlier	Dying at same time	Dying later	Living when other died	Total
Normal pigs	1	12	13	11	1	38
Experimental young	2	19	10	10	0	41

Here again the shifting of the figures to the left is strong indication of increased susceptibility among the experimental young.

An analysis of the results among the experimental young bred under conditions differing slightly from the normal showed that young of bucks but little affected by the injection showed no evidence of increased susceptibility while marked results were obtained with the young of bucks decidedly affected by the injection. As in the previous sections of this paper the effect of the injection is judged by the local reaction, the change in weight and by the excess of antitoxin in the toxin-antitoxin mixture. Table XLIII shows that in whatever way the effect of the injection upon the buck is measured there is a marked shifting of figures to the left when the buck is severely affected, while in spite of slight differences in condition of breeding the young of bucks but little affected by the injection give results closely approximating to normal.

TABLE XLIII.

Dividing the result of the injection of toxin into experimental young according to the severity of the effect of the injection upon the bucks.

	Number of pigs under observation				
	Dying when control lived	Dying earlier	Dying at same time	Dying later	Living when other died
Local reaction:					
No swelling	0	6	5	4	0
Large ,,	2	17	14	7	0
Change in weight:					
Gain	0	8	9	5	0
Loss	4	22	18	11	0
Excess of antitoxin:					
Over 20 %	0	5	6	4	0
Under 20 %	3	13	10	4	0

In addition to the above figures for single toxin-antitoxin mixtures results were recorded for the young of bucks receiving toxin-antitoxin mixtures containing excess of horse serum, horse serum alone, toxin alone and toxin into bucks already rendered passively immune by maternal transmission. These are given in Table XLIV.

TABLE XLIV.

Further results divided according to the nature of the injection received by the buck.

	Number of pigs under observation				
	Dying when control lived	Dying earlier	Dying at same time	Dying later	Living when other died
Mixture containing excess of horse serum ...	2	5	5	0	0
Horse serum alone ...	1	6	1	4	0
Toxin alone	0	2	2	0	0
Toxin into passively immune pig	1	5	1	0	1
Total	4	18	9	4	1

The results thus far recorded show that when conditions of breeding among the experimental pigs were identical with those of normal pigs there is evidence of increased susceptibility among the young of injected bucks, and also that when the experimental young, bred under conditions differing slightly from the normal, are compared among themselves the young of severely affected bucks are more susceptible than those of bucks unaffected by the injection and the latter give results closely approximating to the normal.

Analysis of results also shows that, as might be expected, the effect is of a more or less transient character and that after a certain interval of time has elapsed since the injection of the father the young are of normal resistance. In Table XLV the results are divided according to whether the young were born within or after 12 months from the time of the injection.

TABLE XLV.

Contrasting the results upon the young of injected bucks born within 12 *months of the injection of the parent with those born during the next year.*

	Number of pigs under observation				
	Dying when control lived	Dying earlier	Dying at same time	Dying later	Living when other died
Simple toxin-antitoxin mixture causing swelling.					
Within 12 months	2	13	8	3	0
After 12 ,,	0	4	6	4	0
Simple toxin-antitoxin mixture causing loss in weight.					
Within 12 months	2	14	9	3	0
After 12 ,,	2	8	9	8	0
Simple toxin-antitoxin mixture with less than 20 °/₀ excess of antitoxin.					
Within 12 months	2	9	4	0	0
After 12 ,,	1	4	6	4	0
Mixture containing excess of horse serum.					
Within 12 months	2	2	1	0	0
After 12 ,,	0	3	4	0	0

The other groups were only tested within 12 months of the injection of the parent, but among the bucks injected with horse serum alone some were put to breed with normal does at once and the results of the young born within three months of the injection of the parent gave

1 dying when control lived 3 ,, earlier than controls injected with a higher dose 2 ,, ,, ,, ,, the same ,, 1 ,, at same time as ,, ,, ,, ,,	6 pigs out of 7 more susceptible than normal.
0 ,, later than the controls	None less susceptible.

There is strong evidence here that the young of bucks injected with horse serum possess less than normal resistance to diphtheria toxin. A few experiments on guinea-pigs to which alcohol had been administered appeared to show that this also has some effect in lowering the resistance of the offspring to diphtheria toxin. It is thus probable that the results dealt with in this section of the paper are of a non-specific character.

The condition of increased susceptibility in the offspring is, therefore, obviously to be referred to some effect on the male reproductive cell, whether by direct action of the toxin, etc. upon the germ cell, or indirectly by a lowering of the vitality of the animal producing it.

H. *Effect of injections upon the rate of breeding of guinea-pigs and the vitality of their young.*

In this section we have accumulated results which show the effect of the injection of the parents upon their rate of breeding and also upon the vitality of their young. In Part 1, Table XVII, we recorded an instance in Cages 100*a* and 100*b*, two other illustrations are now given as follows:

1. Toxin and antitoxin mixtures with different proportions of antitoxin were injected at the same time into three pregnant does.

1 Test dose of toxin			+ 1·1 units of antitoxin			—Still-births.
1	,,	,,	+ 1·3	,,	,,	—Young born alive but died within a few days of birth.
1	,,	,,	+ 1·5	,,	,,	—Young survived.

2. Alcohol was injected into breeding bucks on three or four successive days. A few c.c. were injected, the animals exhibiting severe symptoms of intoxication on each occasion. Four bucks were placed with normal does immediately after the last injection. If both animals in each pair had been normal we would have expected births in all cases within three months, in these cases births occurred at the end of three months in only one case, seven months, and nine months in two others, while the fourth buck was discarded after nine months and the doe was still not pregnant.

So many factors influence the rate of breeding of guinea-pigs that it is necessary when recording results in tabular form to compare only those animals that have been kept under identical conditions.

In the following tables only those pigs are recorded that were kept under the same conditions in the same section of the animal house. In all cases the parents were injected when 250 grams in weight and were placed in the breeding runs before they reached 400 grams. Under these conditions a large majority of normal uninjected pigs commence to breed in five months after they have reached 250 grams in weight. The second litter follows usually three months after the first. Tables XLVI and XLVII give the average number of months elapsing between the injection of the parent and the birth of the first and second litters

respectively for injected does paired with normal bucks. The results are divided according to the nature of the injection and the change of weight resulting. The separation of the toxin-antitoxin injection into two groups according to whether excess of horse serum was present in the mixture seemed advisable in consideration of the results in Part 2, where it was found that cases of lowered resistance were frequent when a large volume of horse serum was contained in the neutral mixture. The figures appearing under "Toxin into immune young" refer to passively immune does injected with toxin and subsequently used for breeding second generation pigs such as are recorded in Tables XXXIII, XXXIV, and XXXV of Part 2.

TABLE XLVI.

Showing the average time elapsing between injection of mother and birth of first litter of young divided according to change in weight produced by the injection.

Nature of injection	Months elapsing between injection and birth of first litter		Number of does under observation	
	Increase in weight	Decrease in weight	Increase in weight	Decrease in weight
Toxin and antitoxin	7·6	7·9	16	11
Toxin and antitoxin (excess of serum)	6·2	7·7	12	10
Toxin into immune young	6·9	9·2	4	11

TABLE XLVII.

Showing the average time elapsing between injection of mother and birth of second litter of young divided according to change in weight produced by the injection.

Nature of injection	Months elapsing between injection and birth of second litter		Number of does under observation	
	Increase in weight	Decrease in weight	Increase in weight	Decrease in weight
Toxin and antitoxin	10·6	11·6	12	8
Toxin and antitoxin (excess of serum)	8·9	11·1	13	10
Toxin into immune young	9·0	11·5	4	6

It will be seen that in all cases breeding was delayed beyond the average time for normal pigs of the same weight (five months for first litter and eight months for second). This was slightly more marked when a decrease in weight resulted from the injection.

It was noticed that a considerable proportion of still-births occurred among groups of severely affected animals. Table XLVIII was prepared to demonstrate this point. We must emphasize here the

identity of conditions of breeding of all pigs included in the tables because our experience in breeding normal guinea-pigs for routine purposes shows that the proportion of still-births is greatly increased by adverse conditions.

TABLE XLVIII.

Showing number of still-births occurring among the various groups of pigs under observation divided according to change of weight occurring after the injection.

		Change of weight of parent					
		Increase in weight			Decrease in weight		
Which parent injected	Nature of injection	Number of still-births	Total births	Percentage number of still-births	Number of still-births	Total births	Percentage number of still-births
Doe only	Toxin & antitoxin	1	66	1·5 %	12	84	14·3 %
Doe only	Toxin & antitoxin (excess of serum)	8	66	12·1	12	78	15·4
Doe only	Toxin into immune doe	0	10	0	18	57	31·6
Buck only	Toxin & antitoxin	3	13	23·1	14	56	25·0
Buck only	Toxin & antitoxin (excess of serum)	—	—	—	8	28	28·6
Buck & doe	Toxin & antitoxin	4	17	23·4	4	17	23·4
		Cage 80 (L0)			Cage 79 (L+)		
Buck & doe	Toxin & antitoxin	7	121	5·8	19	128	14·8

Table XLVIII shows that the proportion of still-births is higher among does severely affected by the injection and that the injection of the male parent also affects the number of still-births. Among the bucks the same restrictions occur that no figures are included unless the conditions of breeding were identical.

It will be seen that the presence of excess of horse serum in the toxin-antitoxin mixture increases the number of still-births, and also that the number is high among the young of does passively immune by maternal transmission which have received an injection of toxin. It is somewhat surprising that this table does not show marked irregularities because so many factors contribute to still-births. They are more prevalent among large litters than small and almost invariably occur in five-pig litters: a single litter of this size would greatly affect the results. However, in Cages 79 and 80 we have a good comparison dealing with fairly high figures; among L0 parents there were 7 still-births in a total of 121, while in the L + group there were 19 in a total of 128.

It was also noticed that as a general rule the young of severely affected pigs took longer to reach the standard weight than those of

animals less affected. To tabulate such results it is necessary to bear in mind that pigs increase in weight much more rapidly in summer than in winter, also that the fewer pigs there are in a litter the heavier they are at birth and the more rapidly they come up to weight. Possibly other factors may contribute such as whether the young are the first that the doe has given birth to, but our figures are too small to enable us to detect such differences. Table XLIX gives the average number of days taken by various groups of pigs to reach 250 grams weight during the summer.

TABLE XLIX.

Showing the rate at which pigs of different sized litters reached the standard weight of 250 *grams in* summer *time divided into various groups according to the nature and effect of the injection into the mother.*

		Average number of days coming up to weight: Number of pigs in litter				Number of pigs under observation: Number of pigs in litter			
Nature of injection	Change of weight	1	2	3	4	1	2	3	4
Toxin and antitoxin	Increase	13·0	26·3	32·9	—	4	12	17	0
	Decrease	18·0	36·7	32·4	—	2	7	27	0
Toxin and antitoxin with excess of serum	Increase	19·5	25·3	36·0	53·5	2	14	18	4
	Decrease	24·5	42·5	31·6	51·2	2	4	15	4
Toxin into immune young	Increase	15·5	—	35·0	—	2	0	9	0
	Decrease	21·0	25·1	36·0	54·6	4	13	3	3

TABLE L.

Showing the rate at which pigs of different sized litters reached the standard weight of 250 *grams in* winter *time divided into various groups according to the nature and effect of the injection into the mother.*

		Average number of days coming up to weight: Number of pigs in litter				Number of pigs under observation: Number of pigs in litter			
Nature of injection	Change of weight	1	2	3	4	1	2	3	4
Toxin and antitoxin	Increase	26·2	45·9	59·0	—	5	12	10	0
	Decrease	25·5	46·2	57·3	44·5	2	4	3	4
Toxin and antitoxin with excess of serum	Increase	35·3	42·2	59·0	—	9	12	7	0
	Decrease	33·0	38·7	55·9	—	1	8	8	0
Toxin into immune young	Increase	—	37·5	—	—	0	6	0	0
	Decrease	—	—	45·0	—	0	0	3	0

It would appear from Table XLIX that in cases of litters comprising one and two pigs the effect of the treatment on the mother is marked, in all cases the young of severely affected does took the longest times

to reach the standard weight. This effect is not noticeable in litters of three or four pigs, nor in the winter time (given in Table L). It is probable that the effect is masked wherever the natural conditions are unfavourable to rapid growth.

Summary—Part 3.

1. A male guinea-pig which has received a single injection of a mixture of diphtheria toxin-antitoxin causing severe constitutional disturbance, may beget offspring of slightly lower resistance than normal to diphtheria toxin.

2. This effect is generally restricted to young born within twelve months after the injection of the father, being rarely noticed in the young of later litters.

3. An increased susceptibility to diphtheria toxin is likewise observed in the offspring of male or female guinea-pigs which have received a large dose of horse serum. The greater susceptibility to diphtheria toxin of the young of male guinea-pigs which have been treated with toxin-antitoxin may therefore be non-specific in character.

4. The injection of diphtheria toxin-antitoxin mixtures into guinea-pigs whether male or female reduces their rate of breeding and lowers the vitality of their young.

5. These effects are most pronounced when the toxin-antitoxin mixture produces severe constitutional disturbance or contains excess of horse serum.

Conclusion.

In reviewing the results recorded in Parts 1, 2 and 3 of this paper, bearing upon some of the problems connected with immunity to diphtheria toxin exhibited in the case of the guinea-pig, we have seen that the maximum immunity response to diphtheria toxin, as indicated by the degree of passive immunity of the young, is obtained when an injection of a mixture of diphtheria toxin and antitoxin contains between 1 and 1·2 units of antitoxin to the test dose of toxin. Such a mixture causes considerable constitutional disturbances as shown by reaction at seat of injection and falling off in weight. The immunity induced becomes less and less as the quantity of antitoxin in excess increases.

When it is borne in mind that in order to produce immunity by the injection of a mixture of toxin and antitoxin a comparatively large dose

of toxin is required, it appears extraordinary that a dose of toxin slightly above the minimum fatal dose confers lasting immunity of a similar order when injected into young which are passively immune by maternal transmission; although a dose of toxin injected into a guinea-pig not possessing this initial passive condition of immunity will not, so far as we know, produce even the slightest degree of immunity. A difference between effects of injection of toxin-antitoxin mixtures in the guinea-pig and those of injection of toxin alone in the passively immune guinea-pig was evidenced by the relationship between the reaction and the degree of immunity produced, since generally the larger the reaction with toxin-antitoxin mixtures, the greater the immunity, while with toxin alone in passively immune guinea-pigs the reverse holds good.

The two cases are obviously different, in the one the toxin meets the antitoxin already circulating in the body, while in the other the interaction takes place at the site of inoculation. The process of combination is therefore probably more gradual in the one than in the other.

A single injection, or even a succession of injections of toxin alone into a guinea-pig not possessing any initial antitoxin, is unable to confer any immunity. Whatever these differences may signify it is clear that in order to give the first stimulus to the production of antitoxin in the body cells of the guinea-pig, it is necessary that some antitoxin be present either in the fluids of the body or introduced at the time with toxin. This is in line with results obtained in connection with the immunisation of horses which we hope shortly to publish.

A matter of considerable interest is the great length of time following the injection of an immunity producing mixture of toxin-antitoxin or of toxin alone in a passively immune pig during which the doe can confer passive immunity upon her young. This may be taken as evidence of circulating antitoxin for several years after the injection and yet the young of such pigs when over two months old rarely possess even a trace of immunity. It may be inferred that the circulating antitoxin in the young is all destroyed or excreted within two months from birth, while the mother that has received the single injection of a toxin-antitoxin mixture continues to produce antitoxin for a considerable time. It is evident that the machinery of side-chain production, having once started, continues in the direction of over production for at least two years after the stimulus has been given.

An injurious influence upon the germ cells is brought into evidence when the father alone has been injected, while injections given to

mothers during pregnancy and suckling likewise influence the young in the direction of increased susceptibility, showing that the materials necessary for the nutrition of the young may likewise be deficient in quality or quantity or in both respects, and it is therefore possible that most or all the cells of the body may suffer to some extent as the result of an injection giving rise to production of immunity. As measured by the resistance of offspring to intoxication indicated by tolerance or non-tolerance of a slightly sub-lethal dose this injurious effect of an injection might appear very small, but when taken in conjunction with other effects observed, such as depression of procreative power, exhibited by average delayed appearance of first litters and increase in percentage of still-births, the injury resulting from such an injection may be considerable.

In the case of the injected mother it was shown that although earlier litters possess immunity, later ones may consist of more than normally susceptible young, thus indicating that whatever antitoxin might still be in circulation in the mother is insufficient to confer any immunity on the young, and the injurious influence of the toxin is then brought into evidence.

It does not appear that this lowered resistance to diphtheria toxin is specific, and it has been seen that similar results have been obtained when the parents have been injected with such foreign substances as horse serum, and possibly after repeated massive doses of alcohol.

We have since demonstrated the existence of circulating antitoxin in injected guinea-pigs whose young can tolerate large doses of toxin, while in no case have we found antitoxin in the blood of normal guinea-pigs. Further work is now being conducted upon this subject, dealing with the amount of antitoxin produced by the injection of toxin-antitoxin mixtures in guinea-pigs and the relationship between the circulating antitoxin and the resistance of the animal and its young to toxin.

In view of the possible application of our results to the human subject we are now investigating whether, under cover of antitoxin introduced or normally existent in the blood, a condition of active immunity may be induced by the injection of a small dose of toxin giving rise to little or no constitutional disturbance. We have already found that differences occur in the normal antitoxin value of the blood of different individuals.